

INOVATIVE *IN VIVO* (*IN OVO*) CAM MODEL TO PREDICT EFFICACY AND MODE OF ACTION OF A NEW ANTITUMOR VACCINE STC-1010 ON HUMAN COLORECTAL ADENOCARCINOMA

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Background Colorectal cancer (CRC) is the second-most deadly cancer. Therapeutic resistance to immuno-oncology drives the need for new treatments. Stimulated tumor cell (STC) vaccine (Brenus Pharma) is composed of selected tumor cell lines, stimulated to overexpress tumor-associated or tumor-specific antigens and neoantigens including resistance factors. Haptenization of these proteins forms an immunogenic complex which stimulate the immune system to recognize and target the patient's tumor cells expressing same resistance factors. We report *in vivo* results of STC-1010 vaccine, on human CRC adenocarcinoma from HT29 cell using chorioallantoic membrane (CAM) assay developed by Inovotion in immune reactive model.

Methods This study was carried out in 2 steps: Firstly, three batches of naïve chicken embryos were stimulated by injections of STC1010 at Embryo Development Day (EDD) 11 and EDD13. At EDD18 of each batch, chicken peripheral blood mononuclear cells (PBMCs) were collected and used as anti-tumor reagent to treat respectively, at EDD 11, EDD 13 and EDD16 chicken embryos xenografted with HT29 cells. Activation of PBMCs was evaluated by IL2 and IL12 secretion quantified by ELISA. At EDD18, i.e., 9 days post-graft, *in ovo* anti-tumor efficacy was evaluated by tumor weight, metastatic invasion (qPCR analysis of human Alu sequence in lower CAM) and quantification of tumor-infiltrating by CD8, CD4, IFN-gamma, Perforin and TNF-alpha.^{1,2}

Results Compared to negative control, STC-1010 vaccine induced: 1) significant increase of IL-12 and IL-2 secretion in peripheral blood during the generation of all three batches of PBMCs, confirming previous results (IL-12: +52%, p=0.0003 ; IL-2: +482%, p=0.0033); 2) a significant expression of IFN-gamma in tumor (+130,83%, p=0.0185); 3) a tendency to increase infiltrating cells: CD4+: +79,2%, CD8+: +29,4% , Perforin: +105,5%, TNFα : +78,63% confirmed by immunohistochemistry and translated into 4) a significant increase of tumor necrosis (p = 0.0267); and 5) a tendency of metastasis regression (-49%); with 6) no embryonic toxicity/mortality (daily evaluation of embryonic viability) induced by STC-1010.

Conclusions This *in ovo* study confirms efficacy of the STC-1010 observed in previous CRC syngeneic models and gives more insight about STC mechanism of action with the activation and maturation of dendritic cells, induction of CD8+ and LTh1 against tumor as the main driver of the response, all without toxicity.

Inovotion's CAM model could be used for indication screening and as a pre-proof-of-concept before syngeneic model study.

REFERENCES

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